Biosensors Made from Carbon and Polymer Composite Micro-electromechanical Systems (MEMS)

Interim Report

ONR Award No. N00014-03-1-0893

for the work performed January 21 – July 21, 2003

Dr. Ryan Giedd, PI
Mr. Matt Curry, Co-PI
Dr. Paul Durham, Consultant
Dr. Norm Dobson, PI of Subcontract

Center for Applied Science & Engineering
Southwest Missouri State University
901 South National Avenue
Springfield MO, 65804

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT DATE	3. DATES COVERED *(From - To)*
21-08-2004	Interim	January 2004 – August 2004

4. TITLE AND SUBTITLE
Biosensors Made from Carbon and Polymer Composite Micro-electromechanical Systems (MEMS)

5a. CONTRACT NUMBER
N/A

5b. GRANT NUMBER
N00014-03-1-0893

5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)
Giedd, Ryan, Ph.D.
Curry, Matthew, M.S.
Durham, Paul, Ph.D.
Dobson, Norm, Ph.D.

5d. PROJECT NUMBER
N/A

5e. TASK NUMBER
I, II, III, IV

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Southwest Missouri State University
901 South National Avenue
Springfield, MO 65804

8. PERFORMING ORGANIZATION REPORT NUMBER
#2

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research Regional Office Chicago
230 South Dearborn, Room 380
Chicago, IL 60604-1595

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSORING/MONITORING AGENCY REPORT NUMBER
N/A

12. DISTRIBUTION AVAILABILITY STATEMENT
Approved for Public Release; Distribution is Unlimited

13. SUPPLEMENTARY NOTES
Research in collaboration with Brewer Science, Inc. (Rolla, MO)

14. ABSTRACT
The goal of this project is to develop a new class of biosensors using carbon and polymer based micro-electromechanical systems (MEMS). This report discusses the progress to date on the individual tasks outlined in the proposal. Ion implanter equipment problems have delayed progress, but repairs on the implanter as well as obtaining outside implantation services has been conducted. Current progress has included investigation and refinement of the step-by-step process involved in the development of the CPMEMS. A method has been developed for successfully attaching antibodies to the carbon polymer material (Au/PSA) where antigen binding has been observed, and we have shown using AFM that the poly-D-lysine used to attach the antibodies to the carbon polymer material has caused some non-specific binding. Work on the antigen collection system has led to an alternate technology method of collection.

15. SUBJECT TERMS
CPMEMS, Microlithography Process, Ion Implantation, Atomic Force Microscope, Electron-Beam Deposition, Unity 400, Antibodies, Antigen Collection (ISAVAC).

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dr. Ryan Giedd
N/A	N/A	N/A	UU	14	19b. TELEPONE NUMBER *(Include area code)* (417) 836-5131

Table of Contents

I. Introduction

Previous work in this project, as was discussed in the first interim report, included efforts in Task I-Device Fabrication and Task II-Antigen Platform Feasibility. A study was completed to engineer the appropriate carbon/polymer/metal cluster compositions that have the microstructure and electrical properties that are critically dependent on vibrations or surface acoustic waves (SAW). The ability to produce micron sized structures with microlithography processes was demonstrated and the patterns were imaged using scanning electron microscopy (SEM). The ability to use atomic force microscopy (AFM) to image the surface of the carbon/polymer/metal cluster material was also demonstrated. Data were produced to provide evidence that validates the methodology for building the antibody microarrays needed to detect specific molecular binding events. An initial design and partial prototype was produced for antigen collection.

Work in the last six months has also been primarily in Task I and Task II, focusing on accomplishing the milestones within each. Within this interim report, progress regarding the fabrication of the carbon and polymer based micro-electromechanical systems (CPMEMS), antigen platform feasibility, and the bio-collection system will be discussed.

II. Description of Technical Research Progress

a. Ion implanter equipment interruption

Critical to the completion of this project and fabrication of the CPMEMS is the ion implanter at SMSU. It is the primary step in creation of the metal mixed ion implanted polymers, which are the base materials for the biosensor CPMEMS. During the past year the ion implanter has been unusable due to equipment problems with the system's acceleration voltage and ion source. When the problem began the ion implanter was not producing a stable beam of ions indicated by large fluctuations (arcs) in the system's acceleration voltage. This problem progressed to the point where it was no longer possible to run the ion implanter at the desired beam current and current density. At lower beam current the system was stable enough to process samples and a number of test samples were made.

The test samples processed at the lower beam currents had extreme differences in electrical characteristics when compared to previous ion implanted materials processed at the typical beam currents, and did not have the desired properties for the biosensor CPMEMS. At lower beam currents, time also becomes an issue. Typical implantation duration is about 2 hours. With the necessity to operate the system at lower beam current, implantation length drastically increased too many of weeks of 8-10 hour days.

The system's problem was investigated and it was determined that a new ion source would be needed. Replacing the ion source however, is a time consuming endeavor due to the purchasing process at the university and the custom nature of the equipment. Since this is a non-standard implanter, an identical replacement ion source is not available. A standard ion source that is readily available with approximately the same characteristics as the original ion source was researched and purchased. The new ion source was then adapted to the system with custom machine work.

The troubleshooting, attempted repair of the current ion source, investigation and purchase of the new source, and the custom fit design work has taken up approximately 75% of the time this interim report covers. At this time we are currently testing the new source to determine if it will accommodate our needs and are concurrently pursuing outside implant services companies to process samples during this down-time.

b. Individual CPMEMS layer fabrication

Ion implanter equipment problems have slowed down progress regarding the fabrication of the CPMEMS structures, but have not stopped it completely. To create ion implanted freestanding bridges or cantilevers, a microlithography process must be used. The following schematic steps depict one such process currently being investigated and refined to create the CPMEMS structures.

Steps one through six depict the deposition and microlithography steps required to electro-deposit gold pillars. Initially a conducting stack is created which consists of a tungsten/titanium alloy (W/Ti – 90%/10%) and gold (Au). The deposition of this stack is described in steps one through three and includes RF magnetron sputtering and thermal evaporation. The conducting stack provides the layers needed to produce a surface for Au to be electro-deposited on. In step four, silicon dioxide (SiO_2) is deposited and subsequently patterned using wet chemical etching. The SiO_2 is used in the fabrication of the CPMEMS structures as an insulating and sacrificial layer. The insulating nature of SiO_2 prevents Au from being deposited in certain unwanted areas during electrodeposition. In step seven the remaining SiO_2 functions as a sacrificial layer to initially support the metal/polymer layer during spin coating and implantation until it is removed in step eight.

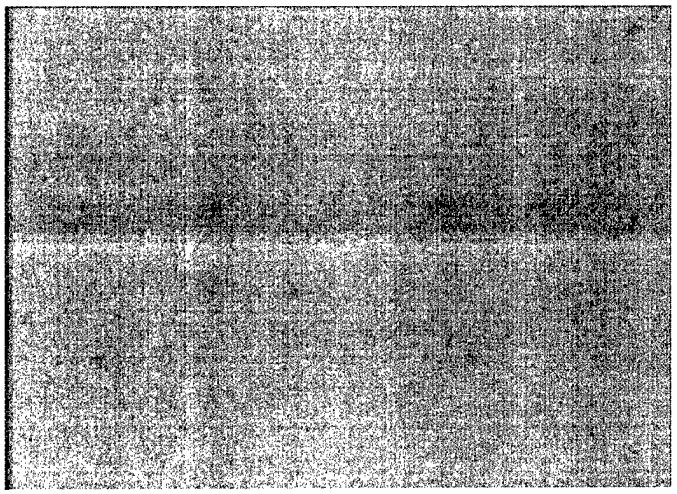
i. Tungsten/Titanium and gold conducting stack

To accomplish the deposition of W/Ti a sputtering target was acquired and deposition of the W/Ti was preformed with radio frequency magnetron sputtering. The sputtering system was calibrated with test runs of varying length to produce a thickness vs. time curve at constant RF power, argon gas flow, and source to substrate distance. The thicknesses of the test substrates were measured with atomic force microscopy and DEKTAK surface profiling. It is now possible to routinely deposit W/Ti on substrates with desired thicknesses. Gold is thermally evaporated and the thickness is monitored during deposition with a quartz crystal monitor so no calibration was needed.

Step 1: Deposit 1,000 Å WTi alloy by RF magnetron sputtering on silicon wafers purchased with silicon nitride deposited on them (~2,000 Å thick)

Side View

Top View

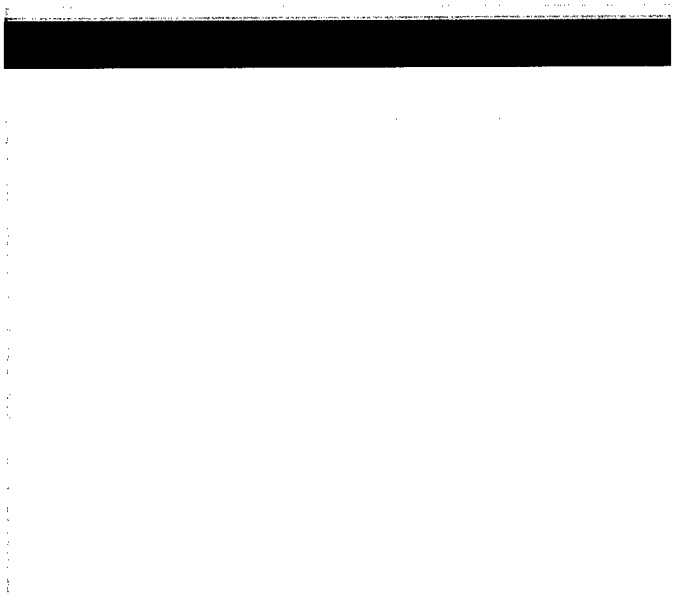


■ $Si + Si_3N_4$ ▩ WTi

Step 2: Deposit 3,000 Å of Au by thermal evaporation

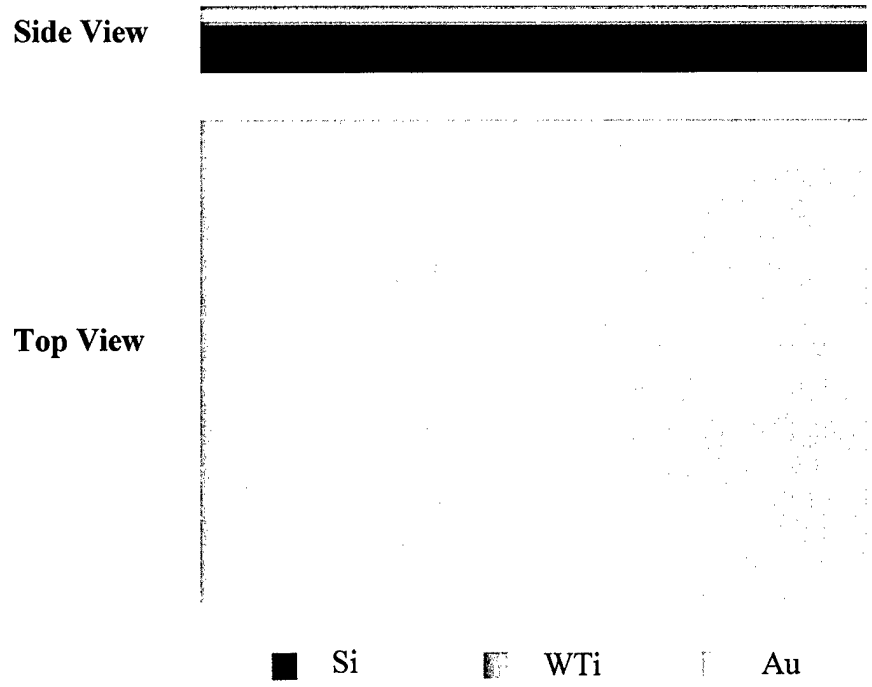
Side View

Top View

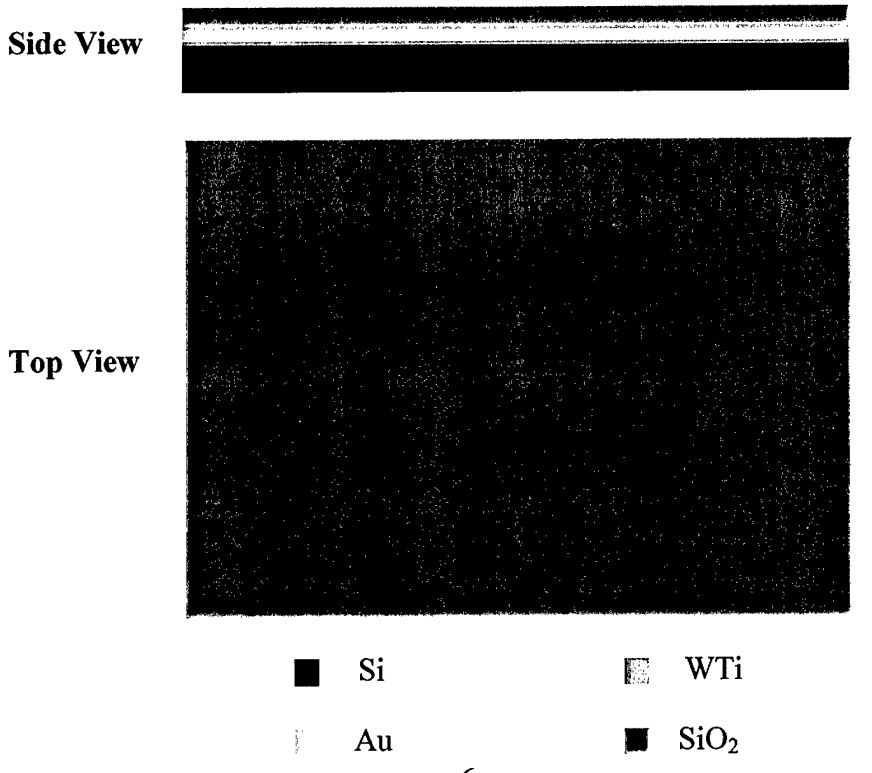


■ Si ▩ WTi ▩ Au

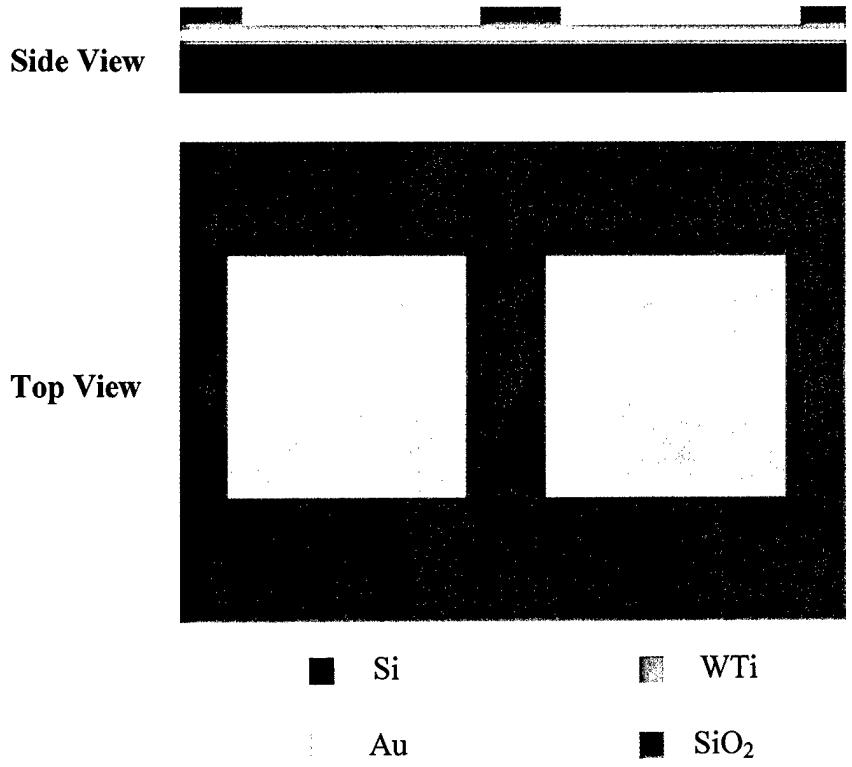
Step 3: Deposit 1,000 Å WTi alloy by RF magnetron sputtering



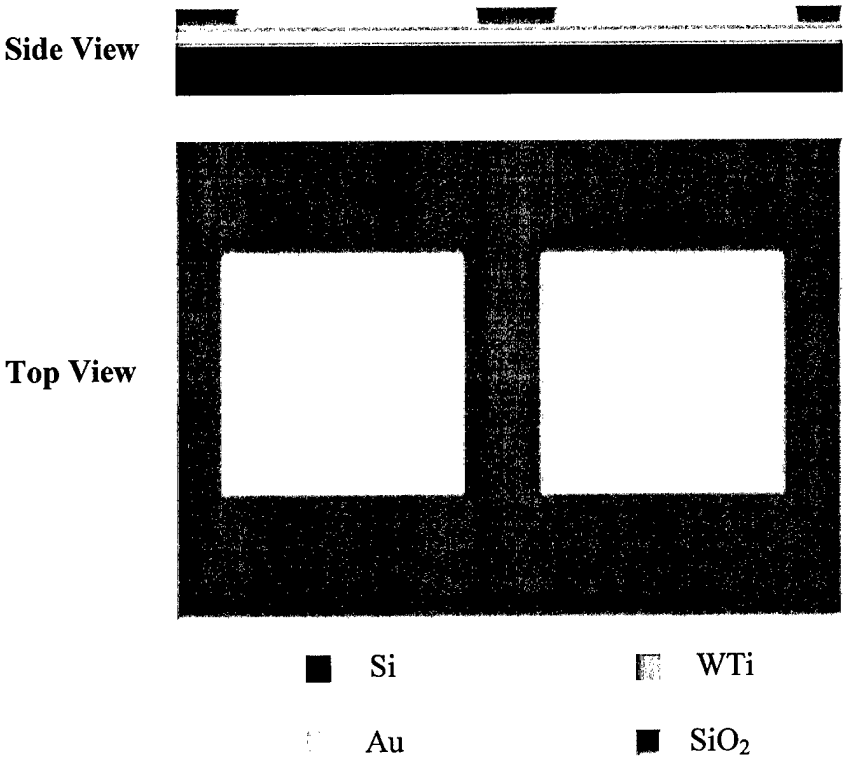
Step 4: Deposit 2.5 μm SiO_2 by RF magnetron sputtering or electron beam evaporation



Step 5: Mask wafer and wet etch SiO_2 layer for addition of Au pillars



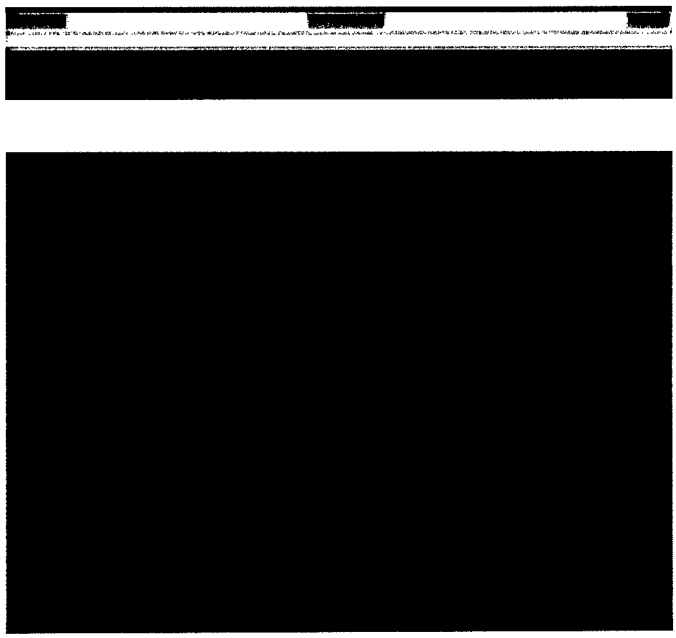
Step 6: Electro-deposit Au to fill in etched SiO_2 regions



Step 7: Spin-coat 1,000 Å PSA and N^+ implant to 1.0×10^{16} ions/cm^2

Side View

Top View



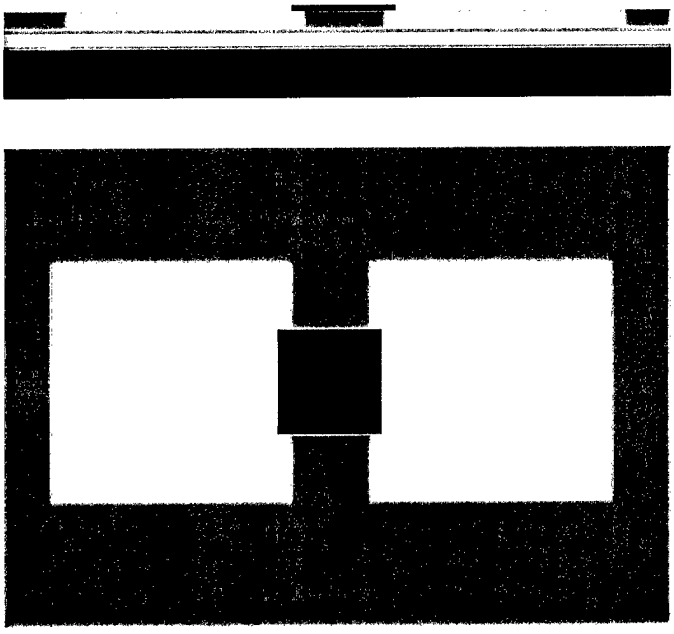
Si SiO_2 PSA

WTi Au

Step 8: Mask wafer and plasma-etch implanted polymer

Side View

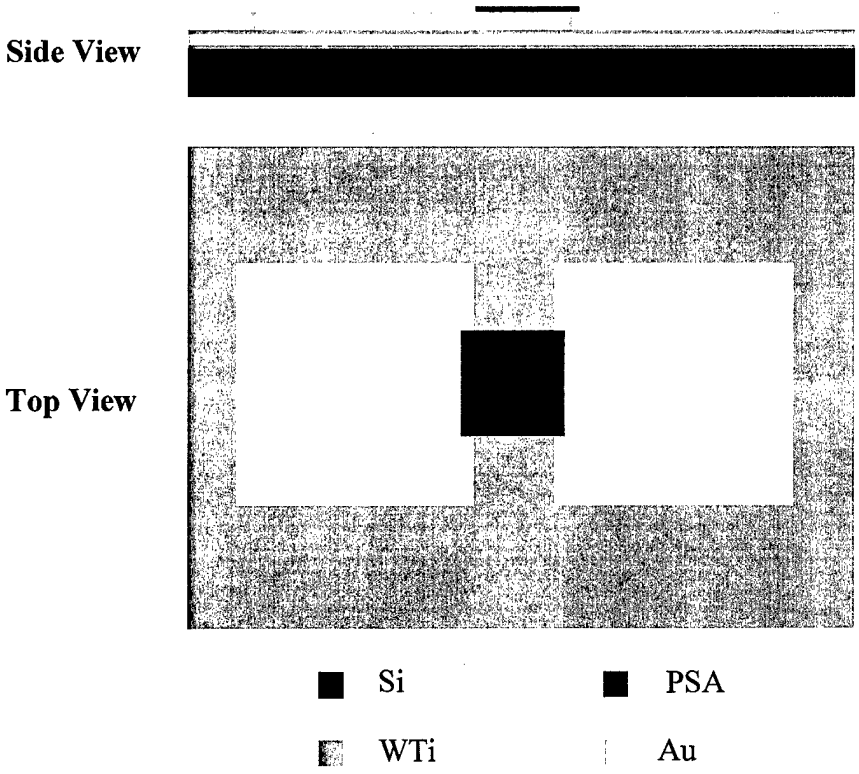
Top View



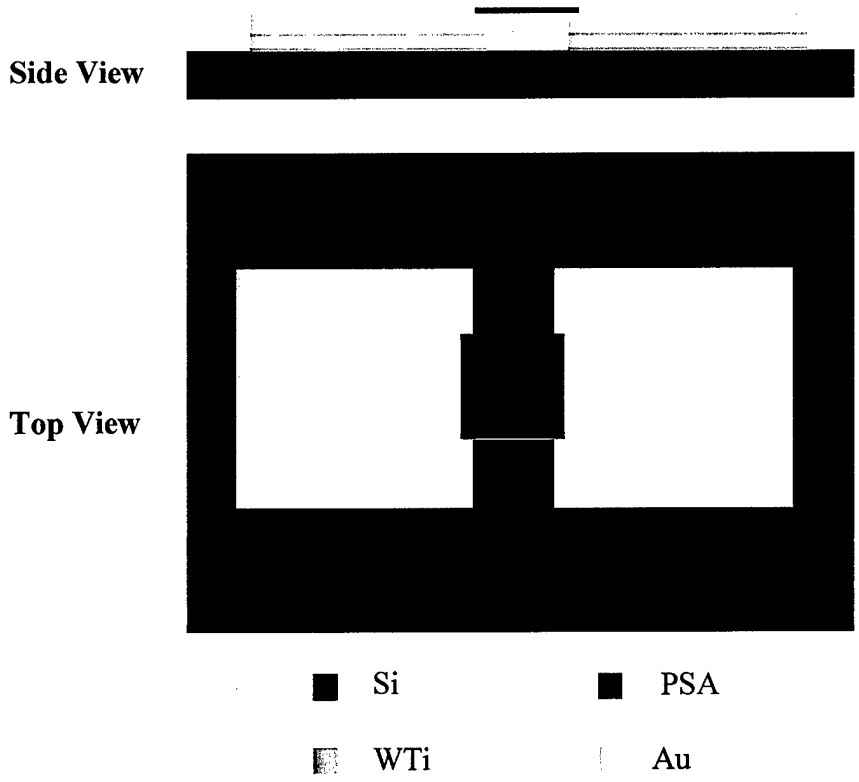
Si SiO_2 PSA

WTi Au

Step 9: Wet etch remaining SiO_2 to create bridge structure



Step 10: Wet etch WTi – Au – WTi stack to isolate pillars



ii. Silicon dioxide

Developments in the area of silicon dioxide (SiO_2) deposition and its subsequent patterning have been made. Our typical method for the deposition of SiO_2 is radio frequency magnetron sputtering and was initially used for SiO_2 deposition. The sputtering system was calibrated with test runs of varying length to produce a thickness vs. time curve at constant RF power, argon gas flow, and source to substrate distance. The thicknesses of the test substrates were measured with atomic force microscopy and DEKTAK surface profiling. It is now possible to routinely deposit SiO_2 on substrates with desired thicknesses.

After the sputtering system was calibrated for SiO_2, a microlithography test mask, with micron sized features, was utilized to formulate a procedure for patterning the SiO_2 layers. SiO_2 was first deposited onto silicon wafers and then using photolithography and hydrofluoric acid wet etching, SiO_2 patterns were produced. Currently the etching times are being optimized to produce the best patterns possible.

The SiO_2 layers processed were on the order of 1,000 to 3,000 Å thick. The sacrificial layer for fabrication of the CPMEMS is 25,000 Å or 2.5 μm. The sputtering system is capable of producing layers 2.5 μm thick, even though it is not designed for such operation. It would take many hours to deposit layers this thick and the sputtering system is not intended for such operation. This led to the investigation of alternate deposition techniques. It was determined that electron beam evaporation would be the most economical and efficient choice for SiO_2 films of this nature. An electron beam evaporation unit was purchased from MDC Vacuum Corporation, and is currently being incorporated into a vacuum system already available at SMSU. Once assembled a complete evaporation system dedicated to SiO_2 deposition will be readily available. Deposition of the 2.5 μm layers with this electron beam system will only take approximately 30 minutes.

iii. Unity 400

As an alternative to SiO_2 a heat dissolvable polymer from Promerus LLC is under investigation by Brewer Science, Inc. (BSI). Instead of using SiO_2 with the requirement of electron beam deposition in step 4, the Unity 400 polymer would be put down via spin coating. Heating the Unity 400 polymer to 425°C will cause it to cleanly decompose and hence create a residue-free air cavity. In step 4 above this polymer would be placed on the wafer instead of the SiO_2. In step 5 the polymer would be masked and instead of wet etching as would be done with SiO_2, this polymer would be Reactive Ion etched. The steps would then be the same as with SiO_2 until step 9, where the Unity 400 would simply be heated to 425°C, which would decompose it to create an air cavity underneath the bridge. Wet etching has been shown to cause stresses on the ion implanted polymer bridge making it bow a little, so there would be and advantage using Unity 400 over SiO_2 due to the different etching techniques used to remove each material.

c. Development of procedure for assembled protein layer

The primary objective of Task II – Antigen Platform Feasibility is to develop the appropriate process to bind proteins, antibodies, or oligonucleotides to the carbon surface of the CPMEMS.

We have developed a method to attach antibodies to the surface of a glass slide which allows binding of a fluorescently tagged antigen (protein) to the antibody. The same methodology was used to attach the antibodies to the Au/PSA mixed carbon-rich surface that will be used to build the CPMEMS bridges. While we can detect antigen binding, there appears to be a problem with non-specific binding of the antigen to the poly-D-lysine that is used to anchor the molecular bridge to the carbon surface. The non-specific binding or background noise could be reduced by inclusion of tween-20 in the wash buffers and increasing the number of washes. Further analysis, using atomic force microscopy (AFM), revealed that the concentration of poly-D-lysine used to coat the surface of the slides was deposited not as a monolayer of the substrate as intended (Figure 1). The thickness of poly-D-lysine would likely increase the amount of background. We are currently testing lower concentrations of poly-D-lysine to determine the minimum amount required for bridge assembly. AFM will be used to observe the thickness of the substrate. In addition, we will test the feasibility of using nitrocellulose as an immobilization substrate since it has been shown to provide a near-quantitative surface, allows for long-term storage of the immobilized antibodies, and allows for proteins (antigens) in solution to interact with the immobilized antibodies.

A major goal of proposed studies was to develop an antibody microarray to efficiently detect hundreds or thousands of proteins in the environment. Towards this end, we have purchased a microarray caster system that will allow us to spot several hundred different antibodies on a single microscope slide. The antigens will be labeled with a fluorescent dye and then incubated with the antibodies on the microarray. The amount of binding will be quantitatively determined using a microarray scanner and accompanying software.

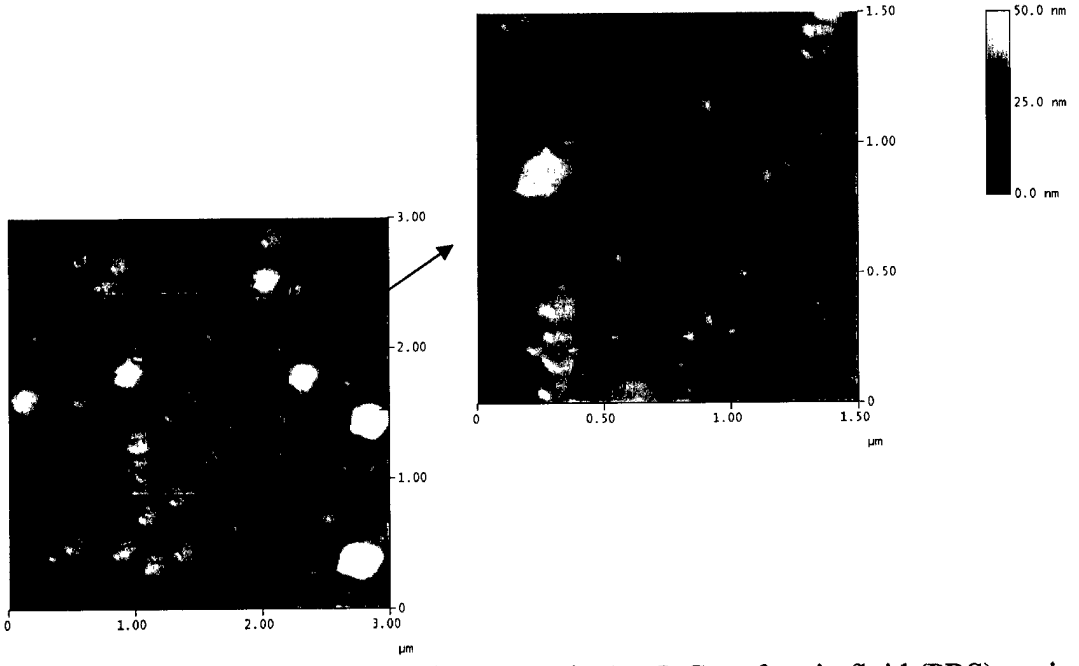


Figure 1: AFM images of poly-D-lysine + protein A + IgG surface in fluid (PBS) environment

d. Developments in the Bio-collection system

Preliminary work on a prototype bio-collection system was discussed in the first interim report. Since that time work in this area has been focused on using alternative technology. An air purification system called ISAVAC was purchased and has been investigated as a means for bio-collection. The ISAVAC system was designed for environmental control by means of airborne particulate removal including pollen, bacteria, smoke, fumes, dust, plant spores, etc. The ISAVAC's area of focus so far has been for use in homes, offices, and hospitals and is installed with the buildings HVAC system.

The ISAVAC is a filter-less system that uses a liquid sub-micron particle collection medium. Water is introduced into the system and then accelerated by an impeller onto an anvil while simultaneously mixed with the intake air from the environment. This creates a dynamic particle removal system where the tiny particles form the environmental air are entrapped in the water and then discharged to a drain. The purified air is then sent into the home or office.

Our interest in this product for this project is not the clean air, but alternately the waste water. This waste water has the potential to contain antigens of interest. Our direction with this product has been one of review, testing, and modification. The system is being reviewed and tested to determine if it is a viable method of bio-collection for the antigens of interest and then modifications will be made to fit our application as needed.

Initial modifications have included changing the once through water system using tap water to a closed cycle system using distilled water and a recirculation pump. The distilled water should eliminate any contamination or interference of any kind from the water source. The closed system or recycled nature of the water supply should serve to provide a concentrated solution. To understand the particle sizes and evaluate this collection system a particle counter capable of measuring liquids will need to be acquired and is currently being researched.

III. Conclusions

a. Accomplishments and future work

During the time frame that this interim report covers we have:

1. Determined that the ion implanter's unstable beam current was a cause of the large fluctuations (arcs) caused by the system's acceleration voltage and ion source, and has therefore been unusable.

2. Acquired a new ion source, which has been retrofitted to the current design of the ion implanter source housing, and have been testing the source at low acceleration voltages.

3. Have sought out other ion implantation services to accommodate the current delay in the CPMEMS research during the downtime of our ion implanter.

4. Investigated the steps involved in the microlithography process to develop the individual CPMEMS layer fabrication, and have concurrently begun refining the process.

5. Demonstrated sputter depositing of WTi to obtain a thickness vs. time curve using DEKTAK surface profilometry to allow for routine WTi deposition at various desired thicknesses as required in the fabrication process of the CPMEMS devices.

6. Demonstrated sputter depositing of SiO_2 to obtain a thickness vs. time curve using DEKTAK surface profilometry to allow for routine SiO_2 deposition at various desired thicknesses as required in the fabrication process of the CPMEMS devices.

7. Been investigating and developing an alternate growth method of SiO_2 such as electron beam deposition apart from the current RF magnetron sputtering method.

8. Began investigation (at Brewer Science, Inc.) on Unity 400 heat dissolvable polymer as an alternative to SiO_2 in the fabrication process of the CPMEMS devices.

9. Demonstrated a method of attaching antibodies to the surface of carbon polymer material (Au/PSA) where it has been shown that antigen binding has occurred, but has also shown that some non-specific binding has also taken place.

10. Shown using AFM that the concentration of the poly-D-lysine used to coat the surface of the carbon polymer material was deposited not as a monolayer as intended and has thus been a cause for the non-specific binding.

11. Investigated an alternate technology (ISAVAC) as an antigen collection system.

In the months ahead, our work will include:

1. Within Task I – Device Fabrication, test the new ion implanter ion source and simultaneously obtain outside services to accommodate for the delay during the implanter's downtime. Continue refining and optimizing the microlithography process to fabricate the CPMEMS devices. Finish development of the electron beam system for SiO_2 growth. Continue investigation of Unity 400 as an alternative to SiO_2.

2. Within Task II – Antigen Platform Feasibility, future studies will involve reducing the non-specific binding or background noise by inclusion of tween-20 in the wash buffers and increasing the number of washes. Also, investigate the feasibility of using nitrocellulose as an immobilization substrate since it has been shown to provide a near-quantitative surface, which allows for long-term storage of the immobilized antibodies, and allows for proteins (antigens) in solution to interact with the immobilized antibodies.

3. Within Task III – Developing Partnerships, efforts will be made, through the Office of Naval Research, to develop partnerships with Department of Defense Laboratories that will enable the testing of the CPMEMS with priority biological agents.

4. Within Task IV – Final Design and Testing, future work will involve the completion of the antigen collection system using alternate technology (ISAVAC).

IV. Notes

1. Y.Q. Wang, M. Curry, E. Tavenner, N. Dobson, and R.E. Giedd, "Ion Beam Modification and Analysis of Metal/Polymer Bi-layer Thin Films", *Nucl. Instrum. Meth. B*, 798-803. (2004).

2. E. Tavenner, P. Meredith, B. Wood, M. Curry, R. Giedd, "Tailored Conductivity in Ion-Implanted Polyetheretherketone", *Synthetic Metals*, (2004 submitted).

3. S. R. Mishra, K. Ghosh, R. Patel, M. Weigand, J. Losby, M. Curry, R. E.Giedd, "Novel two-step technique involving ion-implantation for magnetic nanocomposite preparation", *Nucl. Instrum. Meth.*, (2004 Submitted).

4. Adnan Butt, "Designing, Building and Testing of a Polymer Evaporation System", Southwest Missouri State University, Master of Science in Materials Science Thesis, 2004.